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(54) **GLUCAN DRUG DELIVERY SYSTEM AND ADJUVANT.**

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(56) References cited:
**EP-A- 0 015 175**
**US-A- 4 810 646**

**Biotechnology and Bioengeneering, vol. 28, June 1986, John Wiley & Sons, Inc., S. Jamas et al.: "Morphology of yeast cell wall as affected by genetic manupulation of beta (1--6) glycosidic linkage", pp. 769-784**

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**EP 0 476 063 B1**

Patent Abstracts of Japan, volum 8, no. 279 (C-257)(1716), 20 d cember 1980, & JP-A-59148726 (Root Seiyaku K.K.) 25 August 1984

Industrial Polysaccharides: Genetic Engineering, Structure/Property relations and applications 1987, Elsevier cience Publishers B.V., (Amsterdam, NL); S. Jamas et al.: "Controlled biosynthesis of yeast glucan particles", PP. 65-69

## D scripti n

### Background of the Disclosure

Advances in biotechnology and immunology have presented new challenges for obtaining safe and effective drugs, such as vaccines. For example, new generation subunit and antiidiotype antigens yield very safe vaccines; however, these vaccines, in general, provide poor immune stimulation and prophylactic effects. Therefore, an important aspect of any new drug or vaccine formulation is a component that enhances its safety and efficacy by providing a delivery mechanism and, in the case of vaccines, by boosting the immune response to the antigen. Adjuvants can generally be categorized as components that boost the immune response, and as delivery systems that enhance antigen presentation, provide sustained release of the drug or antigen for extended periods, or target the drug or antigen to specific immune cells.

Serious drawbacks exist in many of today's adjuvants and delivery systems. Most are crude preparations of bacterial or plant origin, or oil emulsion systems, the active components and modes of action of which are unknown. In addition, these compounds are usually toxic and cannot be used safely, especially for human applications. Some preparations of the yeast cell wall component, β-glucan, have been shown to provide enhanced resistance to several infectious diseases when given in conjunction with viral vaccines or killed infected cells. Reynolds et al., 1980, Infect. Immunity, 30:51-57; Holbrook et al., 1981, Infect. Immunity, 35:534-546; Benach et al., 1982, Infect. Immunity, 36:947-951. Some of the adverse effects of administering other β-glucan preparations are described by Williams et al. in U.S. Patent 4,761,402. These effects include anaphylaxis, granuloma development, hypotension development and a high degree of acute toxicity.

EP-A-0 015 175 teaches that Pichia or extracts thereof, in particular their glucans, can be used alone as a medicament for non-specific immunostimulation, potentiation of antibiotics and increasing vaccine activity. The preferred yeast is Pichia fermentans. It is taught that the Pichia or extracts thereof are administered separately in addition to antibiotics, to potentiate the latter.

JP-A-59 148 726 discloses a vehicle for slow-releasing drugs which is a water insoluble glucan produced from Streptococcus mutans. This vehicle is mixed and tabletted with an active drug component. The slow release effect ensues because the glucan molecules bond together to form a drug-incorporating matrix during tabletting, and because the glucan at the surface of the tablet becomes a gel when exposed to moisture, retarding diffusion of the drug out of the tablet.

### Summary of the Invention

The invention relates to a novel pharmaceutical composition which is a drug delivery vehicle and which nonspecifically enhances the immune response. The composition comprises whole glucan particles and a pharmacologically active substance, such as a drug or antigen. The drug or antigen can be contained within, uniformly dispersed with, or chemically linked to the whole glucan particles.

Use of whole glucan particles in pharmaceutical formulations can provide, in combination,

(1) the prolonged release of the drug;

(2) longer half-life of the drug by protecting it from proteolytic, hydrolytic and other clearance mechanisms;

(3) targeted delivery of the drug to macrophages; and

(4) stimulation of the immune response,

When the present composition is administered to an individual the entrapped drug is released through the glucan matrix into the physiological environment. Where the drug is an antigen, the β-glucan component simultaneously acts as an adjuvant to the antigen by enhancing the immune response in the individual.

Whole glucan particles are very pure preparations of β-glucan molecules which avoid many of the undesirable side effects associated with less pure preparations. Whole glucan particles retain the in vivo three-dimensional morphology of the yeast cell walls from which they are derived. Thus, the particles are hollow, which allows the drug or antigen to be incorporated into the cavity. Whole glucan particles have a higher water-holding capacity than β-glucans prepared by other methods which disrupt the cell walls. The water-holding capacity can be controlled by modifying the β-glucan structure, for example, by modifying the amount of branching.

By means of th invention, a drug or antigen can be supplied to an individual while simultaneously providing an adjuvant to boost the immune response to the drug or antigen, by administering to the individual a drug contained within (e.g., encapsulated or entrapped), or uniformly dispersed in, or chemically linked to whole glucan particles. For example, a vaccine can be incorporated into a whole glucan particle,

and the particle administered to an individual to protect against an infectious disease, while simultaneously boosting the individual's immune response to the vaccine.

By incorporating drugs or antigens into the intact whole glucan particle, substantial improvements in the administration and efficacy of drug formulations can be gained.

This invention provides a safe, non-toxic vehicle for in vivo drug delivery that also enhances the immune response, enhances drug presentation in vivo and targets the drug to specific immune cells.

## Brief Description of the Figures

Figure 1 is a graph illustrating the release rate of several proteins of varying molecular weight from whole glucan particles.

Figure 2 is a graph illustrating the rate of release of bovine serum albumin (BSA) from whole glucan particles of different permeabilities.

Figure 3 is a bar graph illustrating the effect on release rate by varying amounts of crosslinking of the drug Cytochrome-C with whole glucan particles.

Figure 4 is a graph illustrating the immunological effect (antibody titer) of whole glucan particles mixed with BSA which were administered to a mouse.

Figure 5 is a graph illustrating the immunological effect (antibody titer) of whole glucan particles mixed with BSA which were administered as a booster to a mouse.

Figure 6 is a graph comparing the immunological effect (antibody titer) of whole glucan particles in which BSA is chemically crosslinked to the particles and in which BSA is physically mixed with the particles.

Figure 7 is a graph comparing the immunological effect (antibody titer) of whole glucan particles in which P55 is chemically crosslinked to the particles and in which P55 is physically mixed with the particles.

## Detailed Description of the Invention

The invention relates to a unique pharmaceutical composition for the controlled and/or continuous release of a drug or antigen from a whole glucan particle combined with an immune system enhancement induced by the β-glucan. The composition thus provides a drug delivery and controlled release system which acts as an adjuvant to the drug. The term "adjuvant" as used herein means a substance which is added to a drug product or formulation which prolongs and enhances the action of the drug or active ingredient. For example, in a vaccine formulation, the whole glucan particles provide a vehicle which enhances antigenicity to the vaccine by prolonging its half-life, targeting it to the macrophages or antigen-presenting cells and simultaneously activating these cells.

β-Glucans provide enhanced resistance to infectious diseases by non-specifically activating a host's immune defense system. Activation occurs through interaction with specific β-glucan receptors on monocytes thereby inducing the release of interleukin-1 (IL-1) and other cytokines and cellular mediators. Czop, (1986) Pathology and Immunopathology Research, 5:286-296; Williams et al., 1988, International Journal of Immunopharmacology, 9:261-267.

Compositions of the present invention, comprising a whole glucan particle and a drug or other pharmacologically active substance, can be used to provide, in combination, a drug delivery vehicle, and an adjuvant in the administration of drugs or vaccines, which compositions are safe and efficacious in humans and animals. The compositions are two phase systems comprising whole glucan particles and a drug or an antigen. The compositions provide substantial improvements in the administration and efficacy of drug formulations. These improvements include:

(1) Providing a non-toxic biodegradable carrier with a defined composition and structure;
(2) Providing a delivery vehicle capable of sustained release of the drug or antigen component;
(3) Targeting the drug or antigen component to macrophages; and
(4) Providing a non-specific immunostimulant, with a known mode of action.

This combination of properties has a synergistic effect, thus, the present invention provides a whole glucan particle delivery system that targets the drug or vaccine to macrophages, activates the macrophages, and extends the drug's half-life in vivo by protecting it from degradation (proteolytic or hydrolytic) and rapid clearance, thus resulting in increased potency and efficacy compared with individual formulations of drug, antigen or whole glucan particles.

The terms "whole glucan", "whole glucan particles", "whole β-glucan" or "whole β-glucan particles" as used herein refer to whole β-glucan particles. Whole β-glucan particles are essentially micron-sized hollow spheres composed of a rigid, semi-permeable glucan matrix. Whole β-glucan particles have the ability to

swell in aqueous solutions.

Whole glucan particles are prepared from yeast cells by the extraction and purification of the alkali-insoluble glucan fraction from the yeast cell walls. The yeast cells are treated with an aqueous hydroxide solution, without disrupting the yeast cell walls, which digests the protein and intracellular portion of the cell, leaving the glucan wall component devoid of significant protein contamination, and having substantially the unaltered cell wall structure of $\beta$(1-6) and $\beta$(1-3) linked glucans. A more detailed description of whole glucan particles and the process of Preparing them is described by Jamas et al. in U.S. Patent 4,810,646 and in co-pending patent applications USSN 297,752 and USSN 297,982, now US-A-5,082,936 and US-A-4,992,540.

Whole glucan particles have been shown to activate human monocyte macrophages by the same mechanisms characterized for other $\beta$-glucans. Czop, Pathology and Immunopathology Research, 5:286-296 (1986). A unique feature of the whole glucan particles is that they retain the in vivo 3-dimensional morphology of the yeast cell wall. Whole glucan particles prepared by this method have several advantages over other $\beta$-glucan preparations, such as those described by DiLuzio et al.; in the International Journal of Cancer, 24:773-779 (1979) and Manners et al., in Biochemistry Journal, 135:19-30 (1973): they are highly pure (e.g., have less than one percent (w/w) protein and less than three percent (w/w) chitin and glycogen), they are intact, having a hollow spherical shape which allows agents to be incorporated into the particles and they can be chemically modified (e.g., crosslinked) to regulate the release rate of the encapsulated drug, and the rate of degradation of the $\beta$-glucan matrix. In addition, whole glucan particles can activate macrophage cells, and thus can be used as carriers or transport vehicles for administration of drugs or antigens to an individual, while simultaneously boosting the individual's immune response, thereby enhancing the action of the drug. The whole glucan carrier acts to deliver the drug or antigen directly to macrophages, where it is slowly released, causing a heightened and sustained immune response. Thus, the present composition allows drugs or antigens to be directed or targeted to macrophage cells.

The whole glucan particles are biodegradable, that is, they bioerode over time in a physiological environment. The terms "biodegradable" or "bioerodible" as used herein are defined as the property or characteristic of a body of microporous material to innocuously distintegrate or break down as a unit structure or entity over a period of time, in response to the biological environment by one or more physical or chemical degradative processes, for example by enzymatic action, hydrolysis, dissolution. The erosion rate may be controlled by varying the ratio of $\beta$(1-6):$\beta$(1-3) linkages in the $\beta$-glucan matrix or by crosslinking.

The drugs suitable for use in the present composition are biologically active substances. These substances include biologically active polypeptides, antigens and vaccines. Any of the drugs used to treat the body can be incorporated in the present composition. The term "drug" is used herein in its broadest sense, as including any composition or substance that will produce a pharmacologic response. Suitable drugs for use with the composition of the invention include without limitation: protein drugs such as insulin; desensitizing agents such as antigens; vaccines such as smallpox, yellow fever, distemper, cholera, fowl pox, antivenom, scarlet fever, diphtheria toxoid, tetanus toxoid, whooping cough, influenza, rabies, mumps, measles and poliomyelitis; antibiotics, such as penicillin, tetracycline, neomycin and erythromycin; antiallergenics; steroids; decongestants; anticholinesterases; sedatives; tranquilizers; estrogens; humoral agents; antipsychotics; antispasmodics; antimalarials; antihistamines; cardioactive agents; nutritional agents such as vitamins, amino acids and fats. Other drugs having the same or different physiological activity as those recited above can be employed in drug delivery systems within the scope of the present invention. Suitable mixtures of drugs can also be incorporated into the composition in lieu of a single drug.

Drugs can be in various forms, such as uncharged molecules, components of molecular complexes, or pharmacologically acceptable salts, such as hydrochloride, hydrobromide, sulfate, phosphate, nitrate, borate, acetate, maleate, tartrate and salicylate. For acidic drugs, salts of metals, amines or organic cations (e.g., quaternary ammonium) can be used. Simple derivatives of the drugs (such as ethers, esters, amides), which have desirable retention and release characteristics, but which are easily hydrolyzed at body pH or by enzymes can be used.

The amount of drug incorporated in the drug delivery device varies widely depending on the particular drug, the desired therapeutic effect and the time span for which it takes the glucan matrix to swell, erode or dissolve. A variety of $\beta$-glucan particles are available to provide complete dosage r gimes for therapy for a range of therapeutic or prophylactic treatments, thus, there is no critical upper limit on the amount of drug incorporated into the device. The lower limit will depend upon the activity of the drug and the time span of its release from the device.

The present compositions are produced by causing the whole glucan particle to swell in the presence of a solution of the drug of choice. Various drugs can therefore be incorporated into the particles by natural

diffusion. Once absorbed within the particles, these drugs may be entrapped by removing the solvent, or by precipitation (e.g., by change of pH, ionic environment or solvent). For example, proteins within the particle can be precipitated by adding ammonium sulfate, ethanol or acetone to a solution of drug and whole glucan particles. The outward diffusion or release rate of the entrapped drugs is therefore a function of their rate of dissolution in the environment of use, and their rate of diffusion through the semi-permeable glucan matrix.

Several methods can be used to swell the glucan particles. Generally, an aqueous solution of the drug to be loaded is prepared and added to an appropriate quantity of whole β-glucan particles and the mixture is allowed sufficient time (generally up to six hours) for the particles to swell. The swollen particles are then removed from the solution, and dried, or contacted with another compound to precipitate the entrapped drug.

The release mechanism of the drug from the cavity of the whole glucan particles into the physiological environment is through natural diffusion and/or degradation of the polymeric glucan network. The rate of release of the drug can be controlled by changing the ratio of β(1-6):β(1-3) linkages in the glucan. Methods of modifying and otherwise manipulating the ratio of β(1-6):β(1-3) linkages, thereby altering the properties of the β-glucan matrix, are described in detail by Jamas et al. in U.S. 4,810,646; and in co-pending patent applications; USSN 07/297,752 and USSN 07/297,982, now US-A-5,082,936 and 4,992,540. For example, by chemical, enzymatic or genetic modification of the ratio of β(1-6):β(1-3) linkages, the water-holding capacity and permeability of the whole glucan particle can be changed, thereby controlling the rate of release of the drug incorporated therein. The effect of reducing permeability of the β-glucan matrix on the release rate of bovine serum albumin (BSA) is illustrated in Figure 2. Additionally, the size (e.g., molecular weight) of the drug molecule is important. Larger molecules, such as proteins, will exhibit a slower rate of release *in vivo*, as illustrated in Figure 1. Thus, the properties of the glucan carrier can be tailored specifically to the drug of interest.

The release rate of a molecule from a whole glucan particle can be modified by crosslinking it to the glucan matrix. This technique is particularly useful for low molecular weight agents which would normally diffuse rapidly through the glucan matrix. This can be achieved, for example, by adding a crosslinking agent to the mixture of β-glucan particles and the drug. The effect of crosslinking is shown in Figure 3.

The present composition can be administered in any way commensurate with the result or effect desired from the drug. Such methods of administration include orally, intramuscularly, transdermally, intradermally, intravenously, or via the gastrointestinal tract. The composition can be formulated into a liquid solution, tablet, lozenge, suppository, insert or the like. One of the advantages of the present composition is the degradation *in vivo* of the β-glucan vehicle into non-toxic natural compounds.

The amount of the composition administered to a subject will vary on an individual basis depending upon the drug used, the nature of the treatment or therapy, the type and severity of the symptoms to be treated, the size and physical condition of the individual, and the results sought.

Whole glucan particle compositions have several advantages as immunostimulants compared to alternate materials, such as aluminum hydroxide and glucans prepared by other methods (e.g., Di Luzio et al., *Int. J. Cancer*, 24:773-779 (1979); Manners et al., 1973, *Biochem J.* 135:19-31). Whole glucan particles are more pure than these glucans and retain the *in vivo*, three dimensional morphology of the yeast cell, thereby providing an intact, hollow structure into which drugs can be incorporated. Glucans prepared by other methods are not intact because the processes used include treatments which disrupt the yeast cell walls, and which destroy the unique functional features of whole glucan particles.

The invention is further illustrated by the following Examples.

## EXAMPLE 1

### Method to Incorporate Proteins Into Whole Glucan Particles by swelling and Physical Entrapment

Three proteins of different molecular weight were incorporated into whole glucan particles using the following procedure. Solutions of cytochrome-C (cyt-C; Mw = 14,000 daltons), bovine serum albumin (BSA; Mw = 67,000 daltons) and alcohol dehydrogenase (yeast) (ADH; Mw = 150,000 daltons) were dissolved in deionized water at concentrations of approximately 12 mg/ml. One milliliter of each protein solution was added to 150 mg of whole glucan particles (produced from Baker's yeast Universal Foods, WI) and from *Saccharomyces cerevisiae* strain R4 according to the method described by Jamas et al. in U.S. Patent 4,810,646 in a test-tube and allowed to swell for two hours at room temperature. The tubes were then transferred to a 45 °C oven and allowed to dry for 12 hours. The resulting dried whole glucan particles contained approximately 80 mg protein/gram of particles.

EXAMPLE 2

Sustained Release of Proteins from Whole Glucan Particles

Three proteins were selected to demonstrate the release rate of different sized molecules from whole glucan particles. Cytochrome-C (Mw = 14,000 daltons), BSA (Mw = 67,000 daltons) and ADH (Mw = 150,000 daltons) were loaded into whole glucan particles according to the method described in Example 1. The dried, loaded particles were resuspended in 10 ml deionized water and were agitated at 37 °C. Samples were removed at regular time intervals and assayed spectrophotometrically for released protein. Figure 1 illustrates the diffusion kinetics of cyt-C, BSA and ADH, from the whole glucan particles, compared to the drugs alone. The amount of time to release 50% of the three drugs is shown in Table 1.

Table 1

| Time to Release 50% of Protein ($T_{50}$) from Whole Glucan Particles (Derived from Baker's Yeast) | | |
|---|---|---|
| Protein | Molecular Weight (daltons) | $T_{50}$ (minutes) |
| Cytochrome-C | 14,000 | 31 |
| Bovine Serum Albumin | 67,000 | 80 |
| Alcohol Dehydrogenase | 150,000 | 200 |

These results demonstrate that the release rate of the proteins from the whole glucan particles is related to the molecular weight of the protein.

EXAMPLE 3

Control of Release Rate From Whole Glucan Particles by Modifying the Permeability of the Glucan Membrane

The following experiment was carried out according to the method described in Example 2, except that whole glucan particles having a higher degree of $\beta$(1-6):$\beta$(1-3) branching compared with other yeast strains were used. These altered whole glucan particles were derived from a mutant strain of yeast, *Saccharomyces cerevisiae* R4 (NRRL Y 15903, described in U.S. Patent 4,810,646). The release rate of BSA from whole glucan particles produced from commercial Baker's yeast and the mutant strain R4 according to the procedure described in U.S. Patent 4,810,646, was determined and compared. The results, shown in Figure 2, demonstrate that the lower permeability of the glucan in particles derived from mutant R4 results in longer retention times of the entrapped BSA ($T_{50}$ = 204 minutes) compared with particles derived from Bakers yeast ($T_{50}$ = 82 minutes).

EXAMPLE 4

Control of Release Rate of Small Molecules from Whole Glucan Particle by Chemical Cross linking

Whole glucan particles containing crosslinked cytochrome-C were prepared by first reacting 5 mg cytochrome-C with 2.5 mg of the heterobifunctional crosslinking reagent sulfosuccinimidyl 6-(4'-azido-2'-nitrophenylamino) hexanoate (sulfo-SANPAH) in 1 ml of 10 mM sodium phosphate buffer (pH 7.4) for 16 hours at 25 °C in the dark. One milliliter of the sulfoSANPAH-cytochrome-C conjugate was swelled into the whole glucan particle cavity by mixing with 150 mg of whole glucan particles and incubating at 25 °C for 2 hours in the dark. The sulfo-SANPAH-cytochrome-C conjugate was crosslinked to the whole glucan particles by exposure to bright light. The unreacted sulfo-SANPAH, cytochrome-C and sulfo-SANPAH-cytochrome-C were removed by washing the whole glucan particles in water. The cross linked whole glucan particle-cytochrome-C conjugate was dried and stored at 4 °C.

The release rate of the protein from the particles was determined according to the method described in Example 2. Figure 3 shows that the release rate of cytochrome-C can be reduced to provide greater than 90% retention over a 24 hour period by increasing the amount of crosslinker added to the whole glucan particles containing cytochrome-C.

EXAMPLE 5

Adjuvant Effect of Whole Glucan Particles in Immunization of Mice with BSA

The *in vivo* adjuvant effect of whole glucan particles in mice was demonstrated by an increase in antibody production in response to the antigen, BSA. BSA was incorporated into whole glucan particles as described in Example 1. CD-1 mice were immunized intradermally with a range of doses of whole glucan particles mixed with BSA in phosphate buffered saline. The dosages of whole glucan particles, containing 10 µg BSA per mouse, were: 0µg, 2µg, 10µg, 250µg and 1250µg. BSA (10 µg) alone was used as a control. Anti-BSA antibody titers were determined by ELISA assay two weeks after immunization. Figure 4 shows the antibody titers 2 weeks post-immunization At 3 weeks post-immunization mice were boosted with a second injection of the same dosages, and the antibody titers were determined at two weeks post-boost. Figure 5 shows the antibody titers 2 weeks post-boost.

The results showed that whole glucan particles had a stimulatory effect on anti-BSA antibody production both in the primary and secondary immune responses (Figures 4 and 5). Stimulation was observed at doses as low as 2µg whole glucan particles per animal (approximately 100 µg/kg body weight).

Example 6

Combined Adjuvant and Delivery Properties of Whole Glucan Particles In Mice

Based on the results of Example 5 the combined adjuvant/delivery properties of the whole glucan particles were investigated with a protein antigen (BSA) and a 55-amino acid peptide (P55). BSA and P55 were loaded into the hollow cavity of whole glucan particles and were cross linked as described in Example 4. The cross linked formulations were prepared so that each dose (0.2 ml) consisted of 100 µg whole glucan particles and either 10 µg BSA or 50 µg P55. Formulations containing the same ratios of whole glucan particles and antigen were also prepared just by mixing together the whole glucan particles with BSA or P55. Each formulation was injected into separate groups of 5 mice (subcutaneous administration) on day 1 of the study and the animals were boosted on day 14 with the same formulation, as described in Example 5. The animals were sacrificed on day 26 and serum was collected and analyzed for anti-BSA or anti-P55 antibodies by direct ELISA. Figures 6 and 7 summarize the results.

As observed in Figures 6 and 7, the utilization of the whole glucan particles as combination adjuvants and delivery vehicle resulted in significantly higher antibody titers than simple mixtures of the antigen with the glucan.

**Claims**

**Claims for the following Contracting States : AT, BE, CH, LI, DE, DK, FR, GB, IT, LU, NL, SE**

1. A bioerodible pharmaceutical composition for administration to a mammal comprising :
   a. whole $\beta$-glucan particles, which are hollow spheres comprised of a semi-permeable glucan matrix; and
   b. a drug;
   said drug being contained within the whole $\beta$-glucan particles or chemically linked thereto, which composition, when placed in a physiological environment releases the drug over time into the physiological environment and wherein said whole $\beta$-glucan particles bioerode in the physiological environment, which whole $\beta$-glucan particles stimulate an immune response in the mammal, thereby providing an adjuvant effect.

2. A pharmaceutical composition of Claim 1, wherein the drug is an antigen or antigenic determinant.

3. A pharmaceutical composition of Claim 2, wherein the drug comprises a vaccine.

4. A therapeutic composition according to Claim 1, wherein the whole $\beta$-glucan particles comprise $\beta$(1-6) and $\beta$(1-3) linked $\beta$-glucans, and said drug is an antigen or an antigenic determinant contained within the whole $\beta$-glucan particles or chemically linked thereto.

5. A composition according to claim 1 for use as a vaccine, comprising whole $\beta$-glucan particles containing therewithin a vaccine for continuous release of the vaccine from the whole $\beta$-glucan

particles, said particles being capable of stimulating an immune response in a mammal thereby enhancing the action of the vaccine.

6. A bioerodible pharmaceutical composition according to Claim 1, for administration to a mammal, wherein the whole $\beta$-glucan particles comprise $\beta$(1-6) and $\beta$(1-3) linked $\beta$-glucans which are hollow spheres comprised of a semi-permeable glucan matrix; and said drug is an antigen or antigenic determinant contained within the whole $\beta$-glucan particles or chemically linked thereto;
wherein the whole glucan particles are specific for macrophages and are bioeroded within the macrophages thereby activating said macrophages; and wherein the composition is capable of releasing the antigen or antigenic determinant in or near the macrophages thereby resulting in an enhanced immune response to the antigen or antigenic determinant.

**Claims for the following Contracting States : ES, GR**

1. A method of making a bioerodible pharmaceutical composition for administration to a mammal comprising bringing together whole $\beta$-glucan particles, which are hollow spheres comprised of a semi-permeable glucan matrix, and a drug in solution whereby said $\beta$-glucan particles are swelled and said drug is contained within the whole $\beta$-glucan particles or chemically linked thereto, the resulting composition, when placed in a physiological environment releasing the drug over time into the physiological environment and wherein said whole $\beta$-glucan particles bioerode in the physiological environment, which whole $\beta$-glucan particles stimulate an immune response in the mammal, thereby providing an adjuvant effect.

2. A method of Claim 1, wherein the drug is an antigen or antigenic determinant.

3. A method of Claim 2, wherein the drug comprises a vaccine.

4. A method of Claim 1, wherein as the $\beta$-glucan particles are used whole particles comprising $\beta$(1-6) and $\beta$(1-3) linked $\beta$-glucans which are hollow spheres comprised of a semi-permeable glucan matrix; and as the drug an antigen or an antigenic determinant is chosen.

5. A method of making a composition according to claim 1 for use as a vaccine, comprising bringing together whole $\beta$-glucan particles and a vaccine and causing said $\beta$-glucan particles to swell and incorporate the vaccine therein, to produce a composition for continuous release of the vaccine from the whole $\beta$-glucan particles, said particles being capable of stimulating an immune response in a mammal thereby enhancing the action of the vaccine.

6. A method according to Claim 1, wherein as the whole glucan particles are used $\beta$(1-6) and $\beta$(1-3) linked $\beta$-glucans which are hollow spheres comprised of a semi-permeable glucan matrix; and as the drug an antigen or antigenic determinant is chosen, the whole glucan particles being specific for macrophages and bioeroded within the macrophages thereby activating said macrophages; and wherein the resulting composition is capable of releasing the antigen or antigenic determinant in or near the macrophages thereby resulting in an enhanced immune response to the antigen or antigenic determinant.

**Patentansprüche**
**Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, LI, DE, DK, FR, GB, IT, LU, NL, SE**

1. Eine biologisch abbaubare pharmazeutische Zusammensetzung zur Anwendung bei Mammalia, enthaltend oder bestehend aus:
   a) ganzen $\beta$-Glucan-Teilchen, welche Hohlkugeln sind, enthaltend oder bestehend aus semi-permeabler Glucan-Matrix; und
   b) einem Arzneimittel;
   wobei das Arzneimittel in den ganzen $\beta$-Glucan-Teilchen enthalten oder chemisch damit verbunden ist, und wobei die Zusammensetzung, wenn sie in eine physiologische Umgebung plaziert wird, das Arzneimittel über einen Zeitraum in die physiologische Umgebung freisetzt und worin die ganzen $\beta$-Glucan-Teilchen in der physiologischen Umgebung sich abbauen und in den Mammalia einen Immun-Response und hierdurch einen Adjuvans-Effekt auslösen.

2. Eine pharmazeutische Zusammensetzung nach Anspruch 1, worin das Arzneimittel ein Antigen oder ein Antigen-Determinant ist.

3. Eine pharmazeutische Zusammensetzung nach Anspruch 2, worin das Arzneimittel ein Vaccin enthält oder daraus besteht.

4. Eine therapeutische Zusammensetzung nach Anspruch 1, worin die ganzen β-Glucan-Teilchen β(1-6)- und β(1-3)-verzweigte β-Glucane enthalten oder daraus bestehen und worin das Arzneimittel ein Antigen oder ein Antigen-Determinant ist, welches in den ganzen β-Glucan-Teilchen enthalten oder damit chemisch verbunden ist.

5. Eine Zusammensetzung nach Anspruch 1, zur Anwendung als Vaccin, enthaltend oder bestehend aus ganzen β-Glucan-Teilchen, welche darin Vaccin enthalten zur kontinuierlichen Freisetzung des Vaccins aus den ganzen β-Glucan-Teilchen, wobei die Teilchen zur Stimulierung eines Immun-Responses in den Mammalia in der Lage sind und hierbei die Vaccin-Wirkung verstärken.

6. Eine biologisch abbaubare pharmazeutische Zusammensetzung nach Anspruch 1, zur Anwendung bei Mammalia, worin die ganzen β-Glucan-Teilchen β(1-6)- und β(1-3)-verbundene β-Glucane enthalten oder daraus bestehen, welche Hohlkugeln aus einer semi-permeablen Glucan-Matrix sind oder solche enthalten, und wobei das Arzneimittel ein Antigen oder ein Antigen-Determinant ist, das innerhalb der ganzen β-Glucan-Teilchen enthalten oder chemisch damit verbunden ist; wobei die ganzen Glucan-Teilchen spezifisch sind für Makrophagen und in den Makrophagen biologisch abgebaut werden und hierbei die Makrophagen aktivieren; und wobei die Zusammensetzung in der Lage ist, das Antigen oder Antigen-Determinant in oder nahe den Makrophagen freizusetzen und hierbei eine Verstärkung des Immun-Responses auf das Antigen oder Antigen-Determinant verursacht.

**Patentansprüche für folgende Vertragsstaaten : ES, GR**

1. Verfahren zur Herstellung einer biologisch abbaubaren pharmazeutischen Zusammensetzung zur Anwendung bei Mammalia durch Zusammenbringen von ganzen β-Glucan-Teilchen, welche Hohlkugeln sind und eine semi-permeable Glucanmatrix enthalten oder daraus bestehen, und einem Arzneimittel in Lösung,
wobei die β-Glucan-Teilchen aufgequollen werden und das Arzneimittel darin in den ganzen β-Glucan-Teilchen enthalten oder damit chemisch verbunden ist,
wobei die sich ergebende Zusammensetzung, wenn sie in einer physiologischen Umgebung plaziert wird, das Arzneimittel über einen Zeitraum in die physiologische Umgebung freisetzt und worin die β-Glucan-Teilchen biologisch in der physiologischen Umgebung abgebaut werden und die ganzen β-Glucan-Teilchen einen Immun-Response in den Mammalia stimulieren und hierbei einen Adjuvans-Effekt auslösen.

2. Ein Verfahren nach Anspruch 1, worin das Arzneimittel ein Antigen oder ein Antigen-Determinant ist.

3. Eine Verfahren nach Anspruch 2, worin das Arzneimittel ein Vaccin enthält oder daraus besteht.

4. Eine Verfahren nach Anspruch 1, worin die ganzen β-Glucan-Teilchen als ganze Teilchen eingesetzt werden und β(1-6)- und β(1-3)- verzweigte β-Glucane sind oder enthalten, welche Hohlkugeln sind aus einer semi-permeablen Glucan-Matrix oder diese enthalten und wobei als Arzneimittel ein Antigen oder ein Antigen-Determinant ausgewählt ist.

5. Ein Verfahren zur Herstellung einer Zusammensetzung nach Anspruch 1, zur Anwendung als Vaccin, in dem ganze β-Glucan-Teilchen und ein Vaccin zusammengebracht werden und die β-Glucan-Teilchen zur Aufquellung gebracht werden und das Vaccin darin incorporiert wird, zur Herstellung einer Zusammensetzung zur kontinuierlichen Freisetzung des Vaccins aus den ganzen β-Glucan-Teilchen, wobei die Teilchen in der Lage sind, in Mammalia einen Immun-Response zu stimulieren und hierbei die Vaccin-Wirkung verstärken.

6. Verfahren nach Anspruch 1, worin als ganze β-GlucanTeilchen β(1-6)- und β(1-3)- verbunden β-Glucane eingesetzt werden, welche Hohlkugeln aus einer semipermeablen Glucan-Matrix sind oder eine

solche Matrix enthalten und als Arzneimittel ein Antigen oder ein Antigen-Determinant ausgewählt ist, wobei die ganzen $\beta$-Glucan-Teilchen spezifisch für Makrophagen und in den Makrophagen biologisch abbaubar sind, wodurch die Makrophagen aktiviert werden, und worin die sich ergebende Zusammensetzung in der Lage ist, das Antigen oder den Antigen-Determinanten in oder nahe den Makrophagen freizusetzen und dadurch einen erhöhten Immun-Respons auf das Antigen oder die Antigen-Determinante bewirken.

**Revendications**

**Revendications pour les Etats contractants suivants : AT, BE, CH, LI, DE, DK, FR, GB, IT, LU, NL, SE**

1. Composition pharmaceutique dégradable par érosion biologique destinée à être administrée à un mammifère comprenant
   a. des particules de $\beta$-glucane entier, qui sont des sphères creuses constituées d'une matrice semi-perméable en glucane; et
   b. un médicament;
   
   ledit médicament étant inclus dans les particules de $\beta$-glucane entier ou chimiquement lié à celles-ci, laquelle composition libère progressivement le médicament dans le milieu physiologique lorsqu'elle est placée dans ce milieu ci dans laquelle lesdites particules de $\beta$-glucane entier se dégradent par érosion biologique dans le milieu physiologique, lesquelles particules de $\beta$-glucane entier stimulent la réponse immune chez le mammifère, conférant ainsi un effet adjuvant.

2. Composition pharmaceutique selon la revendication 1, dans laquelle le médicament est un antigène ou un déterminant antigénique.

3. Composition pharmaceutique selon la revendication 2, dans laquelle le médicament comprend un vaccin.

4. Composition thérapeutique selon la revendication 1, dans laquelle les particules de $\beta$-glucane entier sont constituées d'un enchaînement $\beta$(1-6) et $\beta$(1-3) de $\beta$-glucanes, et ledit médicament est un antigène ou un déterminant antigénique inclus dans les particules de $\beta$-glucane entier ou lié chimiquement à celles-ci.

5. Composition selon la revendication 1 utilisée comme vaccin, comprenant des particules de $\beta$-glucane entier contenant un vaccin à l'intérieur destinée à une libération progressive du vaccin à partir des particules de $\beta$-glucane entier, lesdites particules étant capable de stimuler la réponse immune chez le mammifere amplifiant ainsi l'effet du vaccin.

6. Composition pharmaceutique dégradable par érosion biologique selon la revendication 1, destinée à être administrée à un mammifère, dans laquelle les particules de $\beta$-glucane entier sont constituées d'un enchaînement $\beta$(1-6) et $\beta$-(1-3) de glucanes qui sont des sphères creuses composées d'une matrice semi-perméable en glucane; et ledit médicament étant un antigène ou un déterminant antigénique inclus dans les particules de $\beta$-glucane entier ou chimiquement lié à celles-ci; dans laquelle les particules de glucane entier sont spécifiques des macrophages et dégradables par érosion biologique à l'intérieur des macrophages activent ainsi lesdits macrophages; et dans laquelle la composition est capable de libérer l'antigène ou le déterminant antigénique à l'intérieur ou à proximité des macrophages potentialisant ainsi la réponse immune dirigée contre l'antigène ou le déterminant antigénique.

**Revendications pour les Etats contractants suivants : ES, GR**

1. Procédé de préparation d'une composition pharmaceutique dégradable par érosion biologique destinée à être administrée à un mammifère consistant à meure en contact en solution des particules de $\beta$-glucane entier, qui sont des sphères creuses constituées d'une matrice semi-perméable en glucane entier, et un médicament par lequel lesdites particules de $\beta$-glucane entier sont gonflées et ledit médicament est inclus dans les particules de $\beta$-glucane entier ou chimiquement lié à celles-ci, la composition obtenu pouvant libérer progressivement le médicament dans le milieu physiologique lorsqu'elle est placée dans ce milieu et par lequel lesdites particules de $\beta$-glucane entier se dégradent par érosion biologique dans le milieu physiologique, lesquelles particules de $\beta$-glucane entier stimulent

la réponse immune chez le mammifère, conférant ainsi un effet adjuvant.

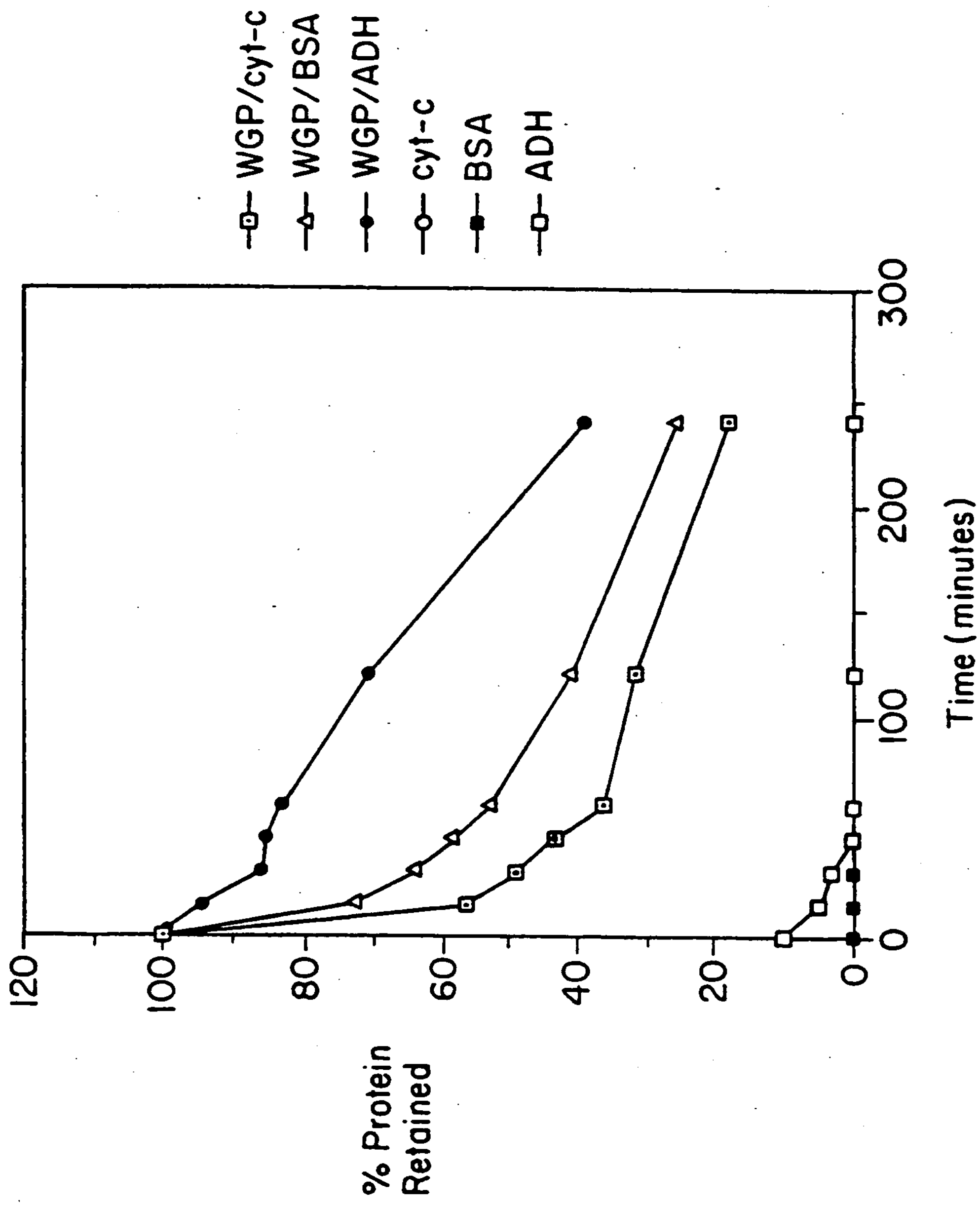
2. Procédé selon la revendication 1, dans lequel le médicament est un antigène ou un déterminant antigénique.

3. Procédé selon la revendication 2, dans lequel le médicament comprend un vaccin.

4. Procédé selon la revendication 1, dans lequel les particules de $\beta$-glucane entier utilisées sont constituées d'un enchaînement $\beta$(1-6) et $\beta$(1-3) de $\beta$-glucanes qui sont des sphères creuses constituées d'une matrice semi-perméable en glucane entier; le médicament choisi étant un antigène ou un déterminant antigénique.

5. Procédé de préparation d'une composition selon la revendication 1 destinée à être utilisée comme vaccin, consistant à mettre en contact des particules de $\beta$-glucane entier et un vaccin et provoquer le gonflement desdites particules et incorporer le vaccin a l'intérieur, en vue de produire une composition à libération continue du vaccin à partir des particules de $\beta$-glucane entier, lesdites particules étant capable de stimuler la réponse immune chez le mammifère amplifiant ainsi l'effet du vaccin.

6. Procédé selon la revendication 1 dans lequel les particules de $\beta$-glucane entier utilisées sont un enchaînement $\beta$(1-6) et $\beta$-(1-3) de glucanes qui sont des sphères creuses constituées d'une matrice semi-perméable en glucane; et le médicament choisi étant une antigène ou un déterminant antigénique, les particules de glucane entier étant spécifiques des macrophages et dégradables par érosion biologique à l'intérieur des macrophages activant ainsi lesdits macrophages; et dans lequel la composition obtenue est capable de libérer l'antigène ou le déterminant antigénique à l'intérieur ou à proximité des macrophages potentialisant ainsi la réponse immune dirigée contre l'antigène ou le déterminant antigénique.



WGP/cyt-c
WGP/BSA
WGP/ADH
cyt-c
BSA
ADH
% Protein Retained
120
100
80
60
40
20
0
0
100
200
300
Time (minutes)


Fig. 1

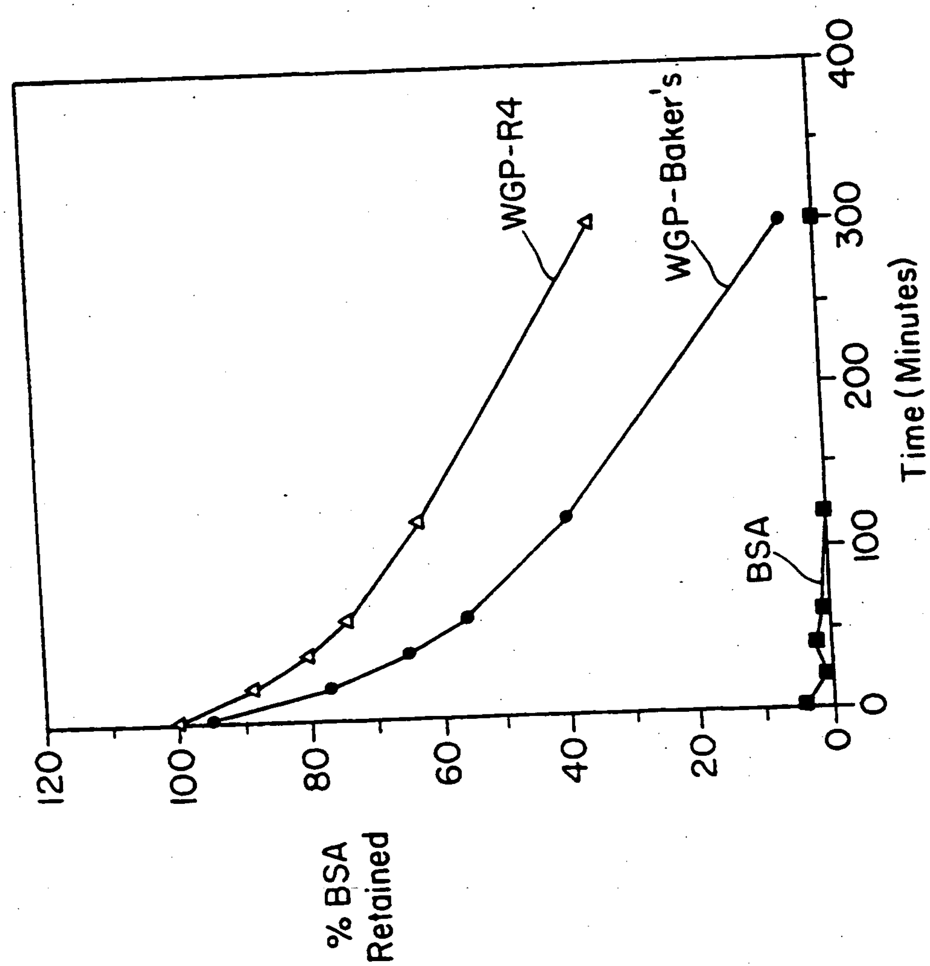

% BSA Retained
120
100
80
60
40
20
0
0
100
200
300
400
Time (Minutes)
WGP-R4
WGP-Baker's
BSA

Fig. 2

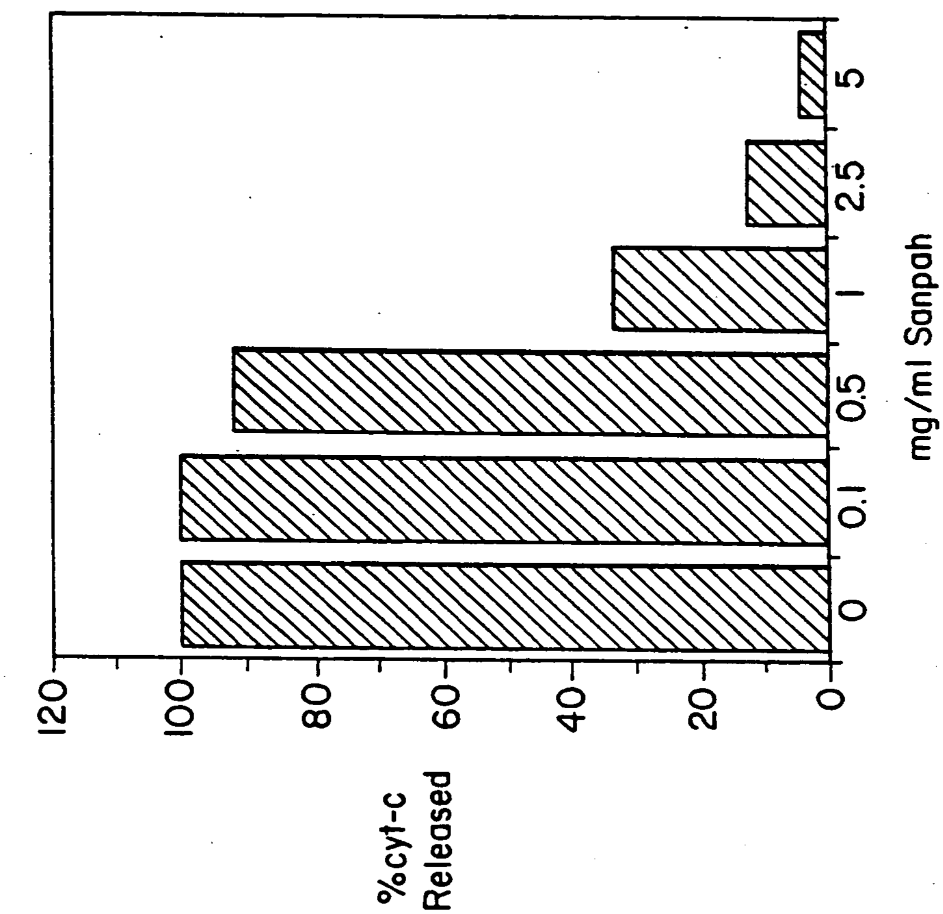
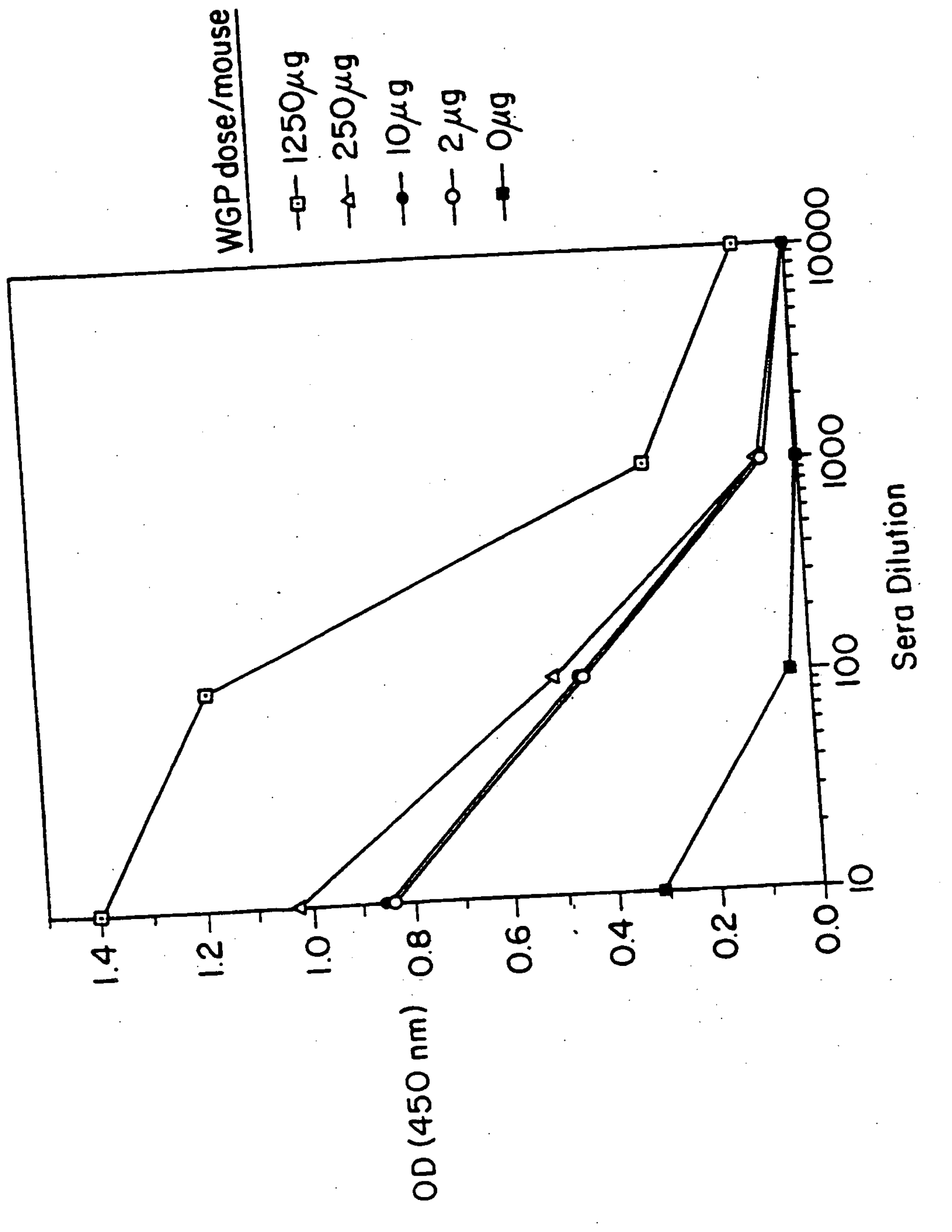



Fig. 3



WGP dose/mouse
1250μg
250μg
10μg
2μg
0μg
OD (450 nm)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10
100
1000
10000
Sera Dilution


Fig. 4

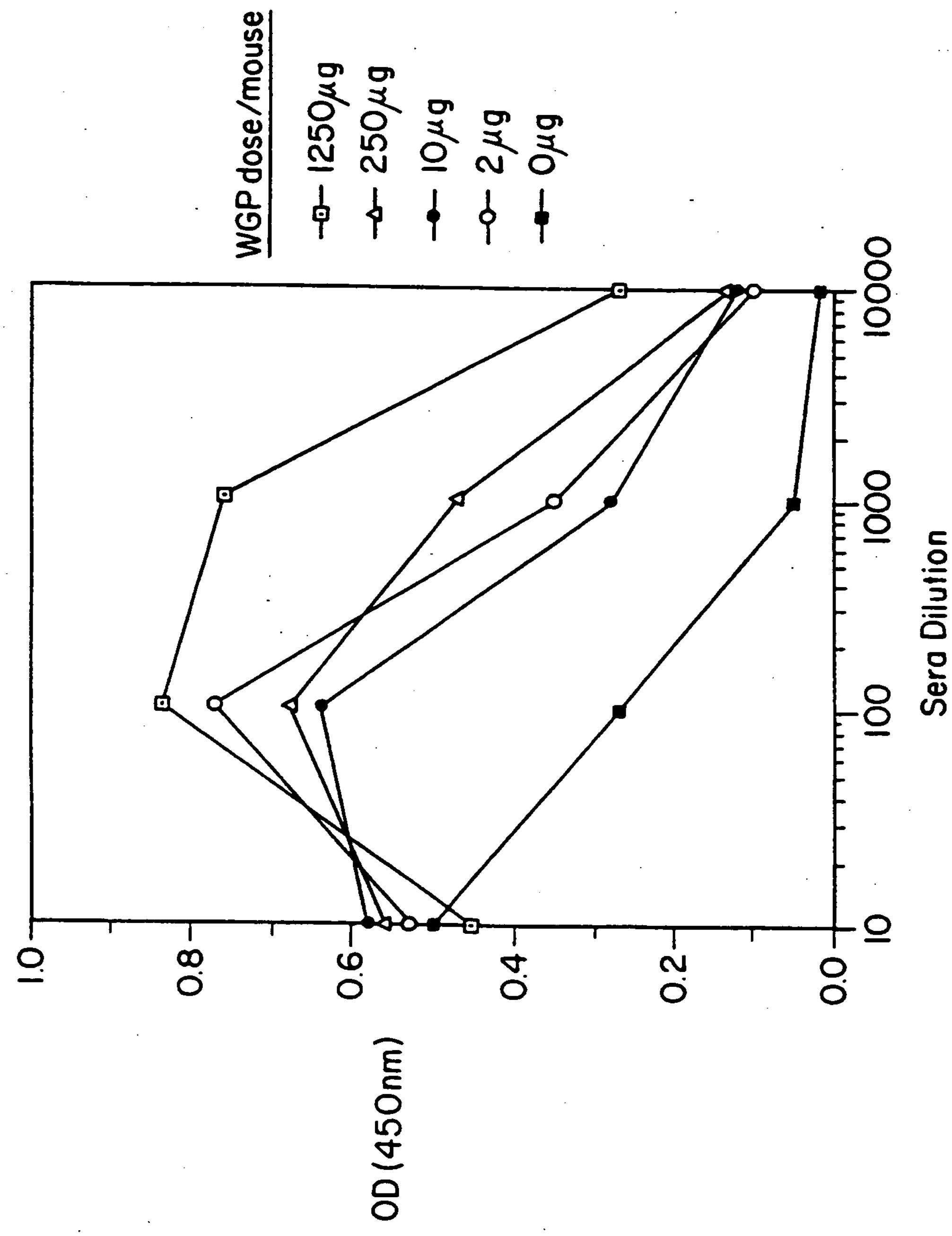

WGP dose/mouse
1250μg
250μg
10μg
2μg
0μg
OD (450nm)
1.0
0.8
0.6
0.4
0.2
0.0
10
100
1000
10000
Sera Dilution

Fig. 5

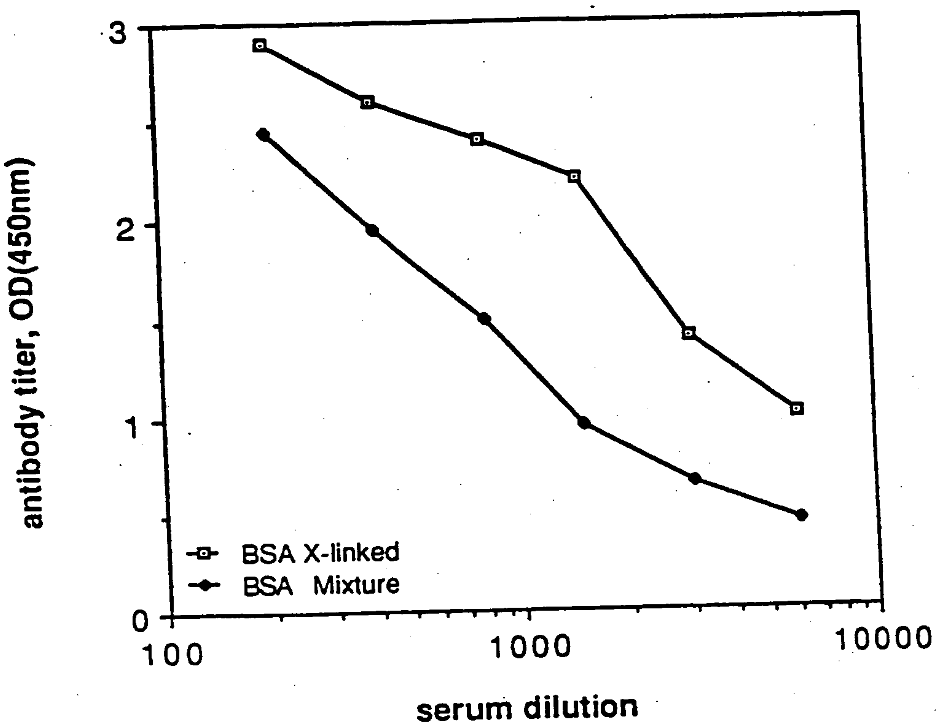

3
2
1
0
antibody titer, OD(450nm)
BSA X-linked
BSA Mixture
100
1000
10000
serum dilution

FIGURE 6

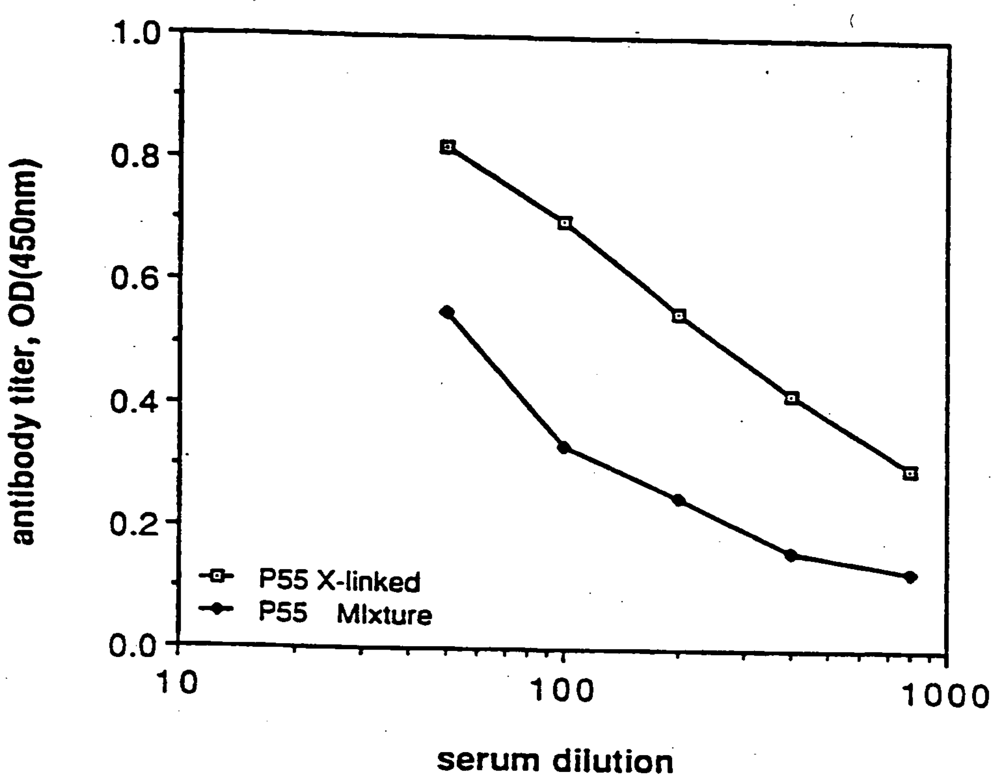

1.0
0.8
0.6
0.4
0.2
0.0
antibody titer, OD(450nm)
P55 X-linked
P55 Mixture
10
100
1000
serum dilution

FIGURE 7